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Effects of SKI 306X, a new herbal agent, on proteoglycan degradation in cartilage explant culture and collagenase-induced rabbit osteoarthritis model

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Summary

Objective: Protective effects of SKI 306X, a natural herbal product extracted from three herbs *Clematis mandshurica*, *Trichosanthes kirilowii,* and *Prunella vulgaris,* on articular cartilage was examined and compared with other osteoarthritis (OA) drugs using *in vitro* and *in vivo* models.

Methods: In vitro culture of rabbit articular cartilage explants was used as a model to measure the effects of drugs on the matrix degradation. The recombinant human interleukin-1α (rhIL-1α, 5 ng/ml) was added to induce proteoglycan (PG) degradation and the degree of PG degradation was assessed by measuring the amount of glycosaminoglycan (GAG) released into the culture medium. In *in vivo* experiment, collagenase was intraarticularly injected twice into the right knee joint of rabbits to induce OA-like change, and test agents were orally administered once a day for 28 days. The degrees of OA-like changes were evaluated through a histological examination.

Results: In vitro study revealed SKI 306X inhibited the degradation of PG in a concentration-dependent manner. *Trichosanthes kirilowii*, which is one of the major components of SKI 306X, also significantly inhibited the GAG release in cartilage explant culture at 0.3 and 0.1 mg/ml. Dexamethasone and NSAIDs, such as diclofenac and rofecoxib, had no significant effects on the suppression of PG degradation. In *in vivo* studies, OA-like degeneration of the articular cartilage and synovial tissue was induced by injecting collagenase into the right knee joint of mature rabbits. At a dose of 200 mg/kg, SKI 306X reduced the OA-like histological changes, whereas diclofenac had no effect at 10 mg/kg.

Conclusion: These results indicate that SKI 306X inhibited PG degradation in cartilage explant culture, and its prophylactic administration significantly protected the knee joint of rabbit from OA-like change in collagenase-induced experimental OA model. This strongly suggests that SKI 306X can be a good OA agent with some cartilage protection activity.

Key words: SKI 306X, Osteoarthritis, Cartilage, Proteoglycan, Collagenase.

Introduction

Osteoarthritis (OA) is known to be induced through several complex mechanisms such as progressive erosion of articular cartilage, proteoglycan (PG) degradation, and disruption of the collagen network, leading to a progressive destruction of joints and functional loss[1]. The aggregating cartilage PG, aggrecan, along with type II collagen, is responsible for the mechanical properties of articular cartilage. Aggrecan molecules are composed of two N-terminal globular domains, G1 and G2, which are separated by an interglobular domain (IGD), followed by a long central glycosaminoglycan (GAG) attachment region and a C-terminal globular domain, G3. Aggrecan monomers interact through the G1 domain with hyaluronic acid and link proteins to form large molecular weight aggregates, which are trapped within the cartilage matrix. Aggrecan provides normal cartilage with compressibility and resilience, and is one of the first matrix components to undergo measurable loss in arthritis. The depletion of these macromolecules results in a decline in the resilience of tissues and the mechanical stresses transmitted across the joint may contribute to the progression of pathological changes in cartilage and subchondral bone. The matrix degradation of articular cartilage appears to be induced by both extrinsic and intrinsic factors. Mechanical disturbances and enzymatic cleavage by matrix metalloproteinases (MMPs) or aggrecanases together with cytokines such as interleukin 1 (IL-1) and tumor necrosis factor α (TNF α) are considered to play critical roles in the degradation of extracellular matrix components[2–5].

Pharmacological management of OA has, up until quite recently, targeted symptoms of the disease rather than the underlying cause; analgesics and non-steroidal anti-inflammatory drugs (NSAIDs) represent the mainstay of treatment[1]. These drugs generally decrease pain and stiffness and improve function, but beneficial effects to the

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underlying cartilage and bone changes have not been demonstrated. Whether NSAIDs are of 'real' benefit to the patient is controversial[6–7]. Recently, attempts have been made by several researchers to develop drugs that reverse, retard or stabilize the underlying pathologic changes in OA, thus providing long-term symptomatic relief[8–10].

SKI 306X is a purified extract from the mixture of three oriental herbs, *Clematis mandshurica, Trichosanthes kirilowii,* and *Prunella vulgaris*, which have been widely used for the treatment of inflammatory diseases such as lymphadenitis and arthritis in Far East Asia[11–12]. SKI 306X is known to have multifunctions such as antiinflammation, immunomodulation, and the activation of blood microcirculation. In a clinical study on patients with OA, SKI 306X was revealed to have a good analgesic efficacy and safety profile and was also suggested to have some cartilage protective activities[13].

In the present study, we investigated the protective effects of SKI 306X on articular cartilage and compared them with those of other OA drugs using *in vitro* and *in vivo* models. For *in vitro* study, rabbit articular cartilage explants were cultured and recombinant human interleukin 1α (rhIL-1α) was used to induce PG degradation. The amount of GAG released into the medium was measured as an index of PG degradation. *In vivo* experimental OA was induced via intraarticular collagenase injection in the knee joint of rabbits, and cartilage destruction and synovial change were histologically evaluated.

Materials and Methods

MATERIALS

Dulbecco's modified Eagle's medium (DMEM), heat-inactivated fetal bovine serum (FBS), and penicillin-streptomycin were purchased from Gibco BRL (Maryland, U.S.A.). Recombinant human interleukin-1α (rhIL-1α) was purchased from R&D systems (Minneapolis, U.S.A.), and the Blyscan proteoglycan & glycosaminoglycan assay kit from Biocolor (Belfast, N. Ireland). Collagenase (*Clostridium histolyticum*, type II; enzyme activity 456 U/mg) was obtained from Sigma (St Louis, U.S.A.). Dexamethasone and Diclofenac sodium were purchased from Sigma and Health Eagle (China), respectively. Rofecoxib was synthesized at the Life Science Research Center of SK Chemicals (Suwon-si, Korea).

PREPARATION AND COMPOSITION OF SKI 306X

SKI 306X was prepared by extracting the mixture of three medical herbs (dried root of *Clematis mandshurica,* dried root of *Trichosanthes kirilowii*, and dried flower and stem of *Prunella vulgaris* at 1:2:1 (w/w), respectively) with 30% (v/v) ethanol in water for 4 h at 80°C. After the extracted solution was filtered and evaporated *in vacuo*, the residue was partitioned between *n*-butanol and water. The *n*-butanol layer was evaporated *in vacuo* and lyophilized for a complete removal of the residual solvent to yield dark-brown powder. SKI 306X was standardized to conform to the regulations imposed by Korea Food and Drug Administration (KFDA).

ANIMALS

Male New Zealand white rabbits were obtained from Damool Science (Taejeon, Korea) and housed individually (MJ-161C, Myung Jin Instrument Co., Korea). All animals had free access to tap water and pellet food (Agribrands, Purina, Korea). The animal experiments were carried out according to the internationally accredited guidelines.

In vitro study

CARTILAGE EXPLANT CULTURES

Articular cartilages from hock joints of 5-week-old rabbits were removed immediately after each animal was sacrificed. The articular cartilage explants were obtained by following the method described by Sandy *et al*.[14–15]. Briefly, after the articular surfaces were exposed surgically under sterile conditions, approximately 200–220 mg articular surfaces per joint were dissected and submerged into complete medium (DMEM, supplemented with heat-inactivated 5% FBS; penicillin 100 U/ml; streptomycin 100 μg/ml). They were then rinsed several times with the complete medium and incubated for 1 to 2 days at 37°C in a humidified 5% CO_2/95% air incubator for stabilization. The complete medium was replaced with a basal medium (DMEM, supplemented with heat-inactivated 1% FBS, 10 mM HEPES, and penicillin 100 U/ml streptomycin 100 μg/ml). Approximately 50 to 60 mg cartilage pieces (2×3×0.35 mm/piece) were placed in 24-well plates and treated with given concentrations of test agents. After pretreatment for 1 h, 5 ng/ml of rhIL-1α was added to the culture medium and further incubated at 37°C in a humidified 5% CO_2/95% air incubator. The culture medium was collected 60 h later and stored at −20°C until assay.

GLYCOSAMINOGLYCANS MEASUREMENTS

The amount of sulphated GAGs in the medium at the end of reaction reflecting the amount of PG degradation was determined by through 1,9-dimethy-methylene blue method using commercially available kit (The Blyscan proteoglycan & glycosaminoglycan assay) according to the instructions of the manufacturer[16].

In vivo study

COLLAGENASE INJECTION FOR OSTEOARTHRITIS INDUCTION

For *in vivo* experiment, collagenase was dissolved in saline at a concentration of 4 mg/ml just before use and the solution was filtrated with a 0.22 μm membrane. Rabbits weighing 2.5–3.0 kg were grouped (N=5) and anesthetized with an intramuscular injection of tiletamine-zolazepam (Zoletil 50®, Virbac, France). After shaving and sterilizing, the right knee joint was injected intraarticularly with 0.25 ml of saline or collagenase solution. The injection was performed twice on days 1 and 4 according to the method of Kikuchi *et al.*[17].

PROTOCOL FOR TREATMENT

SKI 306X (200 mg/kg) and diclofenac sodium (10 mg/kg) were suspended in 0.5% carboxymethylcellulose (CMC) solution and administered orally in a volume of 10 ml/kg using feeding catheter (DJ2-284, Dae Jong Ins. Korea) once a day for 28 days from day 0. Rabbits in control group were given only 0.5% CMC. In this study, we chose the 10 mg/kg dose of diclofenac sodium based on the clinical

dose and its effect on the gastric ulceration in rabbits. In our study, above 20 mg/kg diclofenac showed ulcerogenic effect in rabbits (data not shown).

GROSS OBSERVATIONS

Appearances of the knee joints were observed and body weight changes were measured. They were classified into swelling, reddening, and warmth, and each item was subclassified as mild, moderate, and severe on the basis of severity. Severity of the lameness was also observed and classified into three degrees.

HISTOPATHOLOGICAL EXAMINATIONS

Rabbits were sacrificed at 28 days after collagenase injection. The right knee joints were then dissected and fixed in 10% phosphate-buffered formalin for 2 days and decalcified with Calci-Clear Rapid solution (National Diagnostics, Atlanta, U.S.A.) for 1 week. After decalcification, dorsal sections were collected from the same anatomical site on the femoral condyle and tibial plateau as described by Kikuchi *et al,* processed routinely, and embedded in paraffin[17]. Five micrometer-thin sections were stained with hematoxylin and eosin (H&E) for light microscopic examination. The severity of cartilage lesion was graded through double-blind observations, using the modified histologic scoring system of Kikuchi *et al*.[17]. This system evaluates the severity of lesion based on the loss of superficial layer, erosion of cartilage, fibrillation and/or fissures, disorganization of chondrocytes, loss of chondrocytes, and cluster formation. To detect PG loss in cartilage, duplicate sections were stained with safranin-O method. Articular capsule was also fixed in formalin and stained with H&E for histolopathologic examination. The severity of synovial lesion was graded through the histologic scoring system, based on the hyperplasia of synovial lining cell, hypertrophy of synovial lining layer, infiltration of inflammatory cells, proliferation of granulation tissue, and vascularization[18]. Other parenchymal organs such as stomach, intestine, liver, spleen, and kidney were also histologically examined to investigate possible deleterious effects of the test agents.

STATISTICS

Data were initially evaluated for normal distribution. Statistical significances among groups were tested using Sigma Stat (Jandel Co., San Rafael, CA, U.S.A.) by one way analysis of variance (ANOVA) and Dunnett's test or the Kruskal–Wallis ANOVA and Dunn's test, depending on normality of data. test The significances were further confirmed by the Student's *t*-test or the Mann–Whitney test. Differences were considered signoficnat when P was less than 0.05.

Results

IN VITRO STUDY

Effects on proteoglycan degradation in cartilage explant culture

Rabbit cartilage explants were cultured for 60 h with rhIL-1α (5 ng/ml) in the absence or presence of test agents to examine the protective effects on PG degradation.

Table I
The pharmacological effect of SKI 306X, component herbs, and antiinflammatory agents on GAG release induced by 5 ng/ml rhIL-1α in rabbit articular cartilage explant culture

Agents	Concentration	(*N*)	GAG (μg/mg)
Vehicle		12	1.83±0.28
rhIL-1α		12	4.54±0.59†
SKI 306X	0.3 mg/ml	8	2.02±0.37*
	0.1 mg/ml	8	3.28±0.68
	0.03 mg/ml	8	4.55±0.70
Trichosanthes kirilowii	0.3 mg/ml	8	2.19±0.17*
	0.1 mg/ml	7	2.44±0.20*
	0.03 mg/ml	8	3.93±0.49
Clematis mandshurica	0.3 mg/ml	7	4.53±0.39
	0.1 mg/ml	7	4.98±0.57
	0.03 mg/ml	7	4.89±0.44
Prunella vulgaris	0.1 mg/ml	7	5.09±0.46
	0.03 mg/ml	7	4.92±0.21
Diclofenac	30 μM	8	2.94±0.19
	10 μM	8	3.93±0.43
	3 μM	8	4.41±0.34
Rofecoxib	30 μM	8	4.52±0.49
	10 μM	7	4.88±0.32
	3 μM	7	4.80±0.38
Dexamethasone	10 μM	8	4.23±0.43
	10 nM	7	4.50±0.22

Cartilage explants were incubated for 60 h in DMEM with 1% heat-inactivated FBS and 10 mM HEPES.
Each data represents the mean±S.D.
†P<0.001 vs vehicle, *P<0.05 vs rhIL-1α.

Results were expressed as μg GAG released into the medium per mg wet weight of the cartilage (Table I). When the rabbit cartilage explants were treated with rhIL-1α for 60 h, the amount of released GAG into the culture medium increased significantly compare to the vehicle group (4.54±0.59 μg/mg vs 1.83±0.28 μg/mg).

SKI 306X interfered with the rhIL-1α-mediated degradation of PG in a concentration-dependent manner (P<0.05). SKI 306X at 0.3 mg/ml almost inhibited the PG degradation. *Trichosanthes kirilowii*, one of the components of SKI 306X, also effectively inhibited PG degradation at 0.3 and 0.1 mg/ml. However, the other two components of SKI 306X, *Clematis mandshurica* and *Prunella vulgaris*, did not show any significant inhibitory effects on PG degradation. There might be synergic effects among the three components of SKI 306X since the portion of *Trichosanthes kirilowii* is about 20% (w/w) in SKI 306X and the chondroprotective effect of SKI 306X is not totally explained by the potency of *Trichosanthes kirilowii.* Diclofenac, a nonselective cyclooxygenase inhibitor, showed a tendency to reduce GAG release in a concentration-dependent manner, which was not statistically significant at all of the concentrations. Rofecoxib, a selective COX-2 inhibitor, did not inhibit PG degradation up to 30 μM. Dexamethasone, reported to inhibit the degradation of collagen network at 10 nM, did not inhibit PG degradation at both concentrations of 10 nM and 10 μM[19].

IN VIVO STUDY

Gross observations

Kikuchi *et al.* reported that intraarticularly injected collagenase digests cartilage directly and stimulates an inflammatory reaction in joint tissues at an early stage, after

Table II
Histological evaluation scores of articular cartilage and synovial tissue of rabbits injected with collagenase intraarticularly

	0.5% CMC (*N*=5)	Diclofenac (*N*=5)	SKI 306X (*N*=5)
Tibia condyle			
Loss of superficial layer	3.0±0.7	3.2±0.8	1.8±0.4*
Erosion of cartilage	3.2±0.8	3.0±0.7	1.8±0.4*
Fibrillation and /or fissures	3.0±1.0	2.8±0.8	2.0±0.0
Disorganization of chondrocytes	2.6±0.9	2.4±0.5	1.8±0.4
Loss of chondrocyte	3.2±0.8	2.6±0.5	2.0±1.0
Cluster formation	2.4±0.5	2.4±0.9	1.6±0.5
Sum of score	17.4±3.4	16.4±2.9	11.0±1.6*
Femour plateau			
Loss of superficial layer	3.8±0.4	3.8±0.4	2.4±0.5*
Erosion of cartilage	3.8±0.4	3.8±0.4	2.4±0.5*
Fibrillation and /or fissures	3.6±0.5	3.8±0.4	2.8±0.8
Disorganization of chondrocytes	3.4±0.5	3.8±0.4	2.6±0.9
Loss of chondrocyte	3.4±0.5	3.6±0.5	2.0±1.0*
Cluster formation	3.6±0.5	3.4±0.5	2.2±0.8*
Sum of score	21.6±1.9	22.2±2.4	14.4±4.4*
Synovial tissue			
Hyperplasia of synovial lining cell	3.0±0.6	2.8±0.6	2.2±0.4
Hypertrophy of synovial lining layer	3.1±0.7	2.6±0.8	2.3±0.8
Infiltration of inflammatory cells	3.0±0.4	2.3±0.6	2.0±0.7*
Proliferation of granulation tissue	3.0±0.6	2.2±0.4	1.9±0.8
Vascularization	2.7±0.8	2.0±0.4	1.5±0.7*
Sum of score	14.8±2.7	11.9±2.6	9.9±2.8*

Each data represents the mean±S.D. *P<0.05 compared to 0.5% CMC treatment group.

which the cartilage degeneration proceeds[18]. This experimental OA is a useful animal model, since the cartilage degeneration is similar to the corresponding lesion in human OA. Furthermore, using collagenase at a dose lower than that of papain within a short period can conveniently induce the degeneration. In collagenase-injected group, the swelling, reddening, and warmth of knee joints were the most severe at 1 week after intraarticular injection of collagenase, after which they gradually lessened. Furthermore, they were not significantly different among the collagenase-injected groups, but the number of lame rabbits in the SKI 306X-treated group was less than that of the control group. The body weight decreased during the first week after injection and then gradually increased in collagenase-injected rabbits, and did not decrease in saline-injected group.

Histological examinations

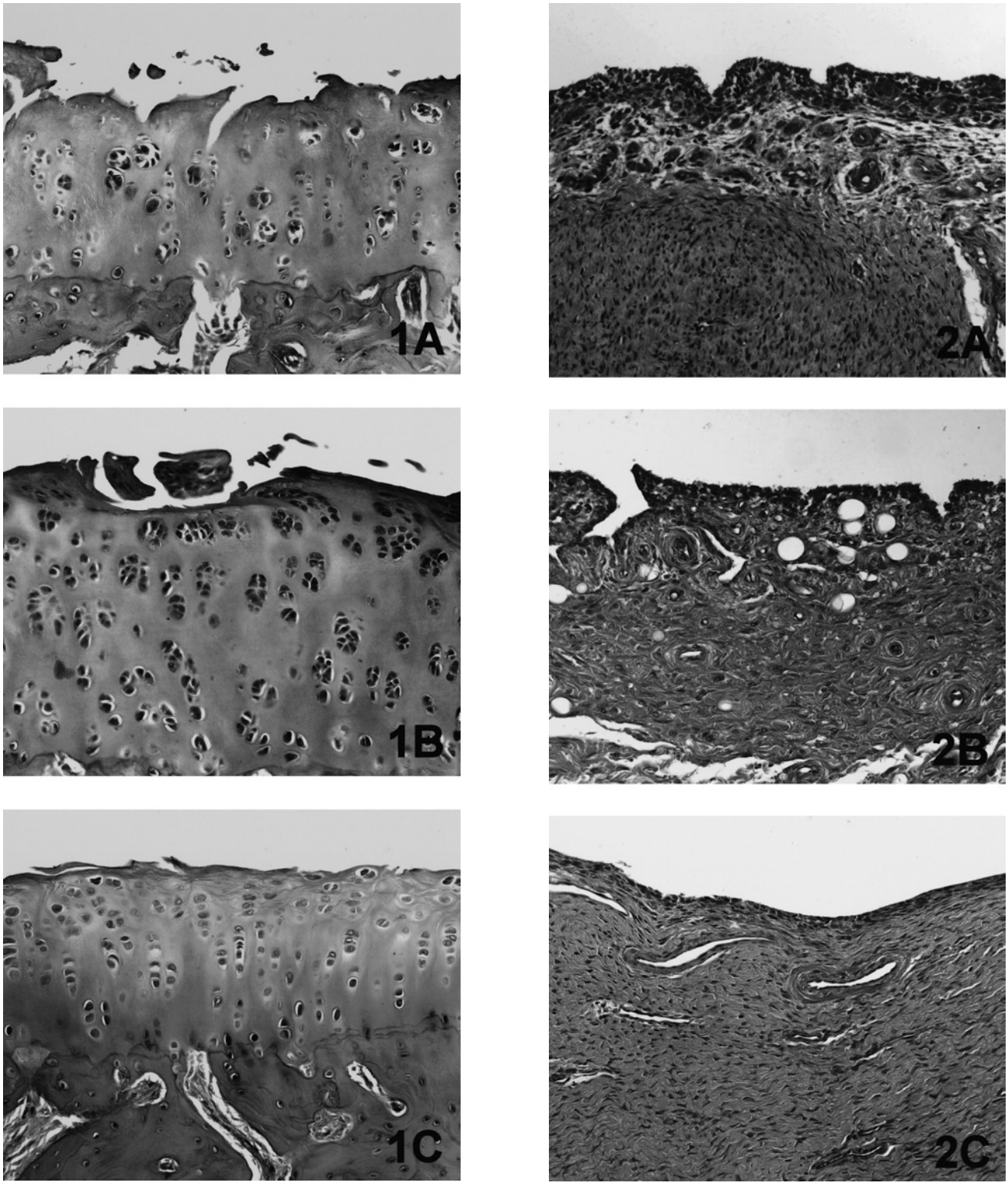
Results of the histopathological evaluation are summarized in Table II. Cartilages of the dorsal section of femoral condyle and tibial plateau were examined by light microscopy. In the saline-injected group, rabbits revealed no significant histological changes in the articular cartilages and synovial tissues (data not shown), whereas all other treated groups developed different degrees of OA-like degenerative changes in the articular cartilages, including loss of the superficial layer, erosion, fibrillation, fissure, disorganization of chondrocytes, and cluster formation. Loss of superficial layer and erosion of cartilage were significantly reduced in the SKI 306X-treated group [Fig. 1(c)] compared to those of the control and diclofenac-treated groups [Fig. 1(a),(b)], respectively. The loss of PG, determined through the safranin-O staining method, was also significantly reduced in the SKI 306X-treated group compared to the control and diclofenac-treated groups in accordance with the results of histopathologic examination [Fig. 3(a)–(d)].

In synovial tissues, hyperplasia and hypertrophy of synovial lining cells, infiltration of inflammatory cells, proliferation of granulation tissue, and vascularization were noted in the control group [Fig. 2(a)]. The degree of synovial changes was similar to that of the collagenase-treated group in the diclofenac-treated group but showed significant alleviation of lesions in the SKI 306X-treated group compared to the control and diclofenac-treated groups [Fig. 2(b),(c), Table II). Global lesion score of the articular cartilage and synovial tissue was significantly lower than those of the control group and diclofenac-treated groups (Table I). However, no significant difference in the global lesion scores was observed between the control group and diclofenac-treated groups, an indication that diclofenac did not exert any significant protective effects. Other parenchymal organs taken from the treated groups did not reveal any remarkable histopathological change. As revealed above, SKI 306X significantly inhibited the progression of collagenase-induced OA-like changes without any observable side effects.

Discussion

Apart from surgical measures, treatment of OA has generally been aimed at alleviating major complaints, such as pain, swelling, and muscle tightness, and thus resulting in improved mobility[1].

Millions of individuals with OA have experienced relief of joint pain and improvement in mobility as a result of taking an NSAID. Despite their efficacy and universal use in OA



Figs 1 and 2. Representative sections of articular cartilage from femoral condyle (left column) and synovial tissue (right column). (1A, 2A) Collagenase-injected group, (1B, 2B) diclofenac treated group, (1C, 2C) SKI 306X treated group. The global lesion score of the articular cartilage and synovial tissue was significantly lower in SKI-306X treated group than control groups, respectively (P<0.05). H&E. ×200.

treatment, the role of NSAIDs in managing OA is controversial due to the negative effects of NSAIDs on cartilages and the frequency and potential severity of side effects (e.g., gastropathy, renal insufficiency, and neurologic complication)[6]. Recently, the discovery of two COX-2 isoforms has provided fresh impetus for the use of NSAIDs in the management of OA agents and development of selective COX-2 inhibitors. The net effects of COX-2 up-regulation

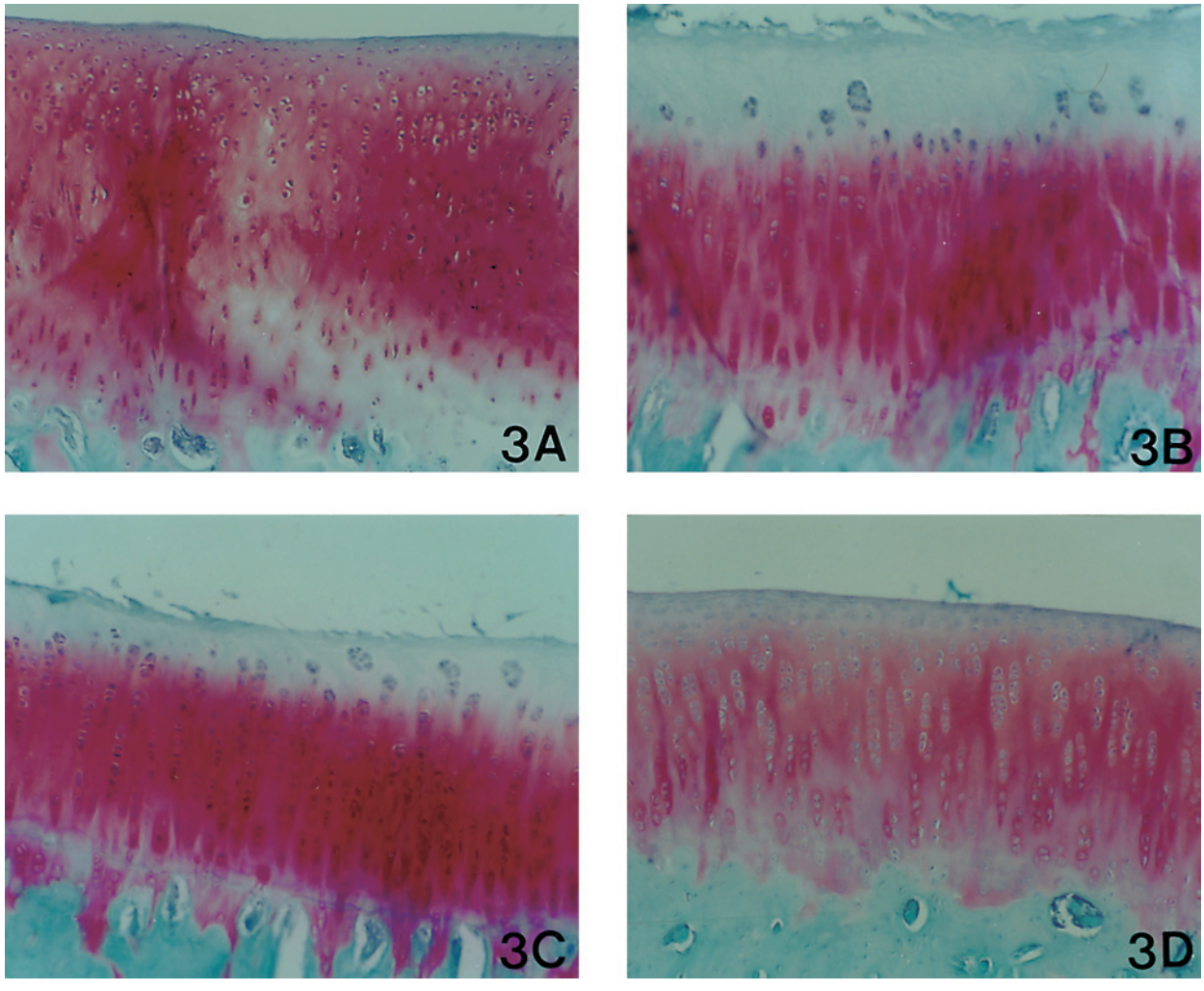


Fig. 3. Representative Safranin-O-stained articular cartilages from femoral condyles. (3A) Normal articular cartilage, (3B) collagenase-injected group, (3C) diclofenac treated group, (3D) SKI 306X treated group. The loss of safranin-O staining in the radial zone was significantly reduced in SKI 306X treated group compared to collagenase-injected group. Safranin O staining. ×200.

and prostaglandin overproduction on cartilage homeostasis are not clear because prostaglandins have multiple direct and indirect (anabolic and catabolic) effects on chondrocytes, which influence cartilage homeostasis[20]. Although selective COX-2 inhibitors may reduce gastro-intestinal troubles, whether these drugs exhibit useful effects on the metabolism of cartilage and bone in OA joints has yet to be determined. For several decades, intraarticular injection of antiinflammatory steroids has been used to relieve the symptoms of OA and to restore articular functions[21]. However, many physicians are reluctant to use steroid injections due to the potential deleterious side effects on cartilage and bones, and the general recommendation is that the same joint should not be injected more than two or three times a year[1]. Therefore, needs are growing for the development of agents that are capable of ameliorating the symptoms of OA by modifying the underlying pathological condition with limited side effects.

Oriental medicinal herbs, which have been widely used for the treatment of various inflammatory diseases such as lymphadenitis and arthritis in traditional Chinese medicine, were selected and screened for their antiinflammatory, analgesic, antiarthritic, and blood microcirculation-enhancing activities and the inhibition of cartilage degeneration enzyme activities. For such purposes, SKI 306X, a new herbal extract, was prepared from a combination of *Clematis mandshurica, Trichosanthes kirilowii, and Prunella vulgaris*[11–12]. Clinical study on patients with OA revealed that SKI 306X not only had a good analgesic efficacy and safety profile, but also showed functional improvements on the limitation of flexion, time taken to go up and down a standard flight of stairs, duration of morning stiffness, and softening of the affected knee joint[13]. Park *et al.* reported that SKI 306X has superoxide anion dismutation effect and its components herbs have hyaluronidase inhibition effects[11]. Because it is well known that reactive oxygen radicals (ROS) and hyaluronidase are implicated cartilage degradation, we thought that SKI 306X might have favorable chondroprotective effects. *In vivo* studies using experimental models have shown that not all NSAIDs affect OA cartilage lesions the same way. In spontaneous murine OA model using C57BL/6 mice, diclofenac reduced the incidence and severity of OA lesions, whereas sodium salicylate and other NASAIDs increased the incidence and severity[22].

In this study, the effects of SKI 306X and its herbal components on cartilage degradation were compared with some representative OA drugs, such as diclofenac

(conventional NSAID), rofecoxib (selective COX-2 inhibitor), and dexamethasone (antiinflammatory steroid)[23–24]. SKI 306X and *Trichosanthes kirilowii*, one of the herbal components of SKI 306X, inhibited the rhIL-1α-induced GAG release in rabbit cartilage explant culture in a concentration-dependent manner. While diclofenac showed some, though not significant, inhibition effects, rofecoxib and dexamethasone revealed none. These results are in agreement with those of others[25–28]. Clay *et al.* reported that neither NSAIDs (indomethacin, tiaprofenic acid, naproxen) nor antiinflammatory steroids (dexamethasone, hydrocortisone, prednisolone) had any significant effects on IL-1β-induced GAG loss from femoral head cartilage in rats *in vitro* even at 100 μM of steroid[25]. There are several reports that diclofenac had no positive effect on cartilage metabolism[26–28]. In *in vivo* study, severe degeneration of the articular cartilage and synovial tissue was induced through the intraarticular injection of collagenase in mature rabbits. At a dose of 200 mg/kg, SKI 306X significantly reduced the degree of OA-like lesions. But diclofenac had no protective effect on cartilage at a dose of 10 mg/kg. This *in vivo* effect together with *in vitro* effects suggests that SKI 306X may protect articular cartilage from degradation.

Chondrocytes produce ROS and the production of ROS by chondrocytes can contribute to degradation of the cartilage matrix[29]. ROS are implicated in both cartilage aging and the pathogenesis of OA, and known to induce apoptosis of synoviocytes *in vitro*[30,31]. Therefore, the chondroprotective effect of SKI 306X may attribute to the antioxidant effect of it. And there is also a possibility that hyaluronidase inhibition effect of SKI 306X may be related. Flannery *et al.* reported that cartilage-derived hyaluronidase activity may implicate the contributing factor for cytokine-induced extracellular matrix degradation during synovial joint disease[32]. However, we cannot exclude other factors such as antagonistic effects on cytokines (IL-1, TNF-α etc.) and catabolic enzymes like MMPs and aggrecanase because the most well characterized enzymatic activities contributing to this process are engendered by MMPs and aggrecanases[33–36]. Further studies will be initiated to investigate the mechanism of action of SKI 306X on MMPs and aggrecanases expression and their enzyme activities. In addition, study on chondroprotective effect of SKI 306X through antioxidant property is in progress.

In summary, SKI 306X inhibited the degradation of PG in a concentration-dependent manner in rabbit explant culture and attenuated the OA-like change induced by intra-articular injection of collagenase in rabbits. This strongly suggests that SKI 306X has the potential to ameliorate the progress of OA by protecting the PG degradation.

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